Independent predictors of cardiac parasympathetic dysfunction in type 2 diabetes mellitus

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ABSTRACT

Introduction: Although the clinical implications of diabetic autonomic neuropathy have been described, the clinical determinants of parasympathetic dysfunction in type 2 diabetes mellitus are not clear. We investigated the clinical determinants of heart rate response to deep breathing in type 2 diabetes mellitus.

Methods: This study involved 207 randomly selected patients with type 2 diabetes mellitus and 141 healthy controls. Heart rate response to deep breathing was measured in all the subjects. Heart rate response to Valsalva manoeuvre and active standing was measured in lesser numbers. Data analysis was done using unpaired Student's t-test, Pearson's correlation test and multiple regression.

Results: Heart rate response to deep breathing, Valsalva manoeuvre and active standing was lower in patients with diabetes mellitus than in controls (p-value is less than 0.0001, 0.01 and 0.01, respectively). Age, female gender and presence of somatic neuropathy were the independent predictors of reduced heart rate response to deep breathing (p-value is 0.001). Independent positive correlation was found between resting heart rate and heart response to deep breathing (p-value is 0.02). Factors associated with depressed heart rate response to deep breathing, but not independently predictive, were duration of diabetes mellitus, presence of hypertension, coronary artery disease, foot ulcer and retinopathy. Mean heart rate response to deep breathing of 47 patients with diabetes mellitus free of all complications was lower compared to controls (p-value is less than 0.01).

Conclusion: Our data suggests that parasympathetic dysfunction mainly coexists with somatic neuropathy. It may be isolated, or precede detection of other complications. Age and female gender are the other predictors of reduced heart rate response to deep breathing in type 2 diabetes mellitus.

Keywords: diabetes mellitus, heart rate variation, parasympathetic dysfunction, somatic neuropathy, type 2 diabetes mellitus

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INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting various organs of the body. In many patients with diabetes mellitus, typical symptoms associated with the disease manifest only after sufficient cumulative effect of diabetes mellitus. As a result, diabetes-related complications and associated clinical conditions may be present by the time it is clinically diagnosed. One such diabetic complication which remains subclinical is autonomic neuropathy. In the light of increased mortality rate associated with diabetic autonomic neuropathy, and its possible involvement in other complications,[1-3] it is crucial to detect early changes in the autonomic tone, as well as its relationship to other complications, for long-term treatment strategies. It is generally believed that the parasympathetic dysfunction manifests much earlier, before the involvement of the sympathetic fibres.[4] In the intact heart, parasympathetic fibres are inhibitory and sympathetic fibres are excitatory. Inhibitory actions of cardiac parasympathetic nerves are reported to provide electrical stability to the heart, thus preventing ventricular tachycardia in humans.[5] There are currently no adequate and comprehensive studies involving all the major clinical characteristics of type 2 diabetes mellitus, and investigating the independent association of each of these clinical characteristics with parasympathetic dysfunction. In the light of this lack of data, this study was undertaken to investigate the independent predictors of parasympathetic dysfunction among the clinical characteristics of type 2 diabetes mellitus.

METHODS

This study was done in patients with type 2 diabetes mellitus attending the outpatient clinic at the Department of Medicine, Kasturba Medical College Hospital. This study was undertaken after obtaiming approval by the institutional ethical committee overseeing human studies.

The study group comprised 207 patients with type 2 diabetes mellitus; they were not selected with respect to age, duration of diabetes mellitus, complications and medication. Study subjects were selected based on established diabetes mellitus, according to ADA criteria. Exclusion criteria were diabetic patients with (1) severe systemic diseases, such as liver disease and kidney failure; (2) heart diseases in which regular sinus arrhythmia was lost; or (3) known neuropathy of other aetiology. The control group comprised 141 healthy subjects. These subjects were free from diabetes mellitus, hypertension, coronary artery disease or any other illness which would hamper with the test results. These comprised volunteers from the hospital staff and their relatives.

All the subjects in the study and control groups were subjected to clinical examination. However, subjects in the study group alone underwent detailed systemic examination. In the personal interviews with the patients, detailed history was obtained with special reference to age, duration of diabetes mellitus, symptoms of neuropathy, diabetes-related complications and medication. In addition to routine general examination, the height and weight of all the subjects were measured. The body mass index was calculated using the formula: weight in kilogrammes (kg) divided by height in metres (m) squared. A 12-lead electrocardiogram was performed in control subjects to rule out any heart disease. In the study subjects, it was done to diagnose any fresh cardiac ischaemic changes. The diverse pattern of clinical manifestations of somatic neuropathy compelled us to adopt the neurological scoring system to define somatic neuropathy. A total score of 0–3 was considered normal. A score of ≥ 4 was considered as having somatic neuropathy (adopted and modified from Kennedy et al).[6] The protocol employed is shown in Table I.

Table I. Protocol employed in systemic examination and scoring of somatic peripheral nervous system.

Neurological examination	Pattern of scoring	Total score
Muscle power*	Grade V = 0 Grade IV = 1 Grade III = 2 Grade II = 3 Grade I = 4 Grade 0 = 5	10
Reflexes:		12
Biceps	Normal = 0	
Quadriceps	Sluggish = 1	
Achilles	Absent = 2	
Sensation*	(Based on degree and	16
Vibration	extension of impairment)	
Pain	Normal = 0	
Temperature	Impaired = 1	
Touch	Severely impaired = 2	

* Upper and lower limbs

Fasting and postprandial blood sugar were measured in all the subjects. They were measured on an autoanalyser using a hexokinase method calibrated with aqueous controls. HbA1c could be measured only in 38 patients with diabetes mellitus, owing to financial constraints. HbA1c was measured by immunoturbidometric inhibition assay. Microalbuminuria was detected by the Albustix or Albym tests in study subjects only. Blood pressure was measured in all the subjects. Two readings were taken five minutes apart in the sitting position. The mean of the two was recorded as blood pressure. The resting electrocardiogram was recorded in a supine position using a standard electrocardiogram. Lead II electrocardiogram was then recorded continuously at a speed of 25 mm/s for 60 seconds. The total number of R-R intervals in one minute was considered as the resting heart rate.

Three tests of parasympathetic function were included in the study. They were heart rate variation with deep breathing (HRV), heart rate response to Valsalva manoeuvre and heart rate response to active standing. HRV was measured in all the 207 type 2 diabetic patients and 141 control subjects. Heart rate response to the Valsalva manoeuvre and active standing could be measured only in 50 diabetic and 30 control subjects. The sympathetic function was tested using blood pressure response to standing. This test was done only in the study group. All the tests were performed in the morning after subjects were completely relaxed. The reproducibility of HRV was investigated in 20 study and 20 control subjects by measuring HRV three times on different occasions over a period of 5–7 days.

HRV was recorded with the subject in supine position, connected to the limb leads of a standard electrocardiogram. Before beginning the test, subjects were taught to breathe at six breaths a minute: five seconds for each inhalation and five seconds for each exhalation. The examiner raised his hand to signal the start of each inhalation and lowered it to signal the start of each exhalation; this was supported by verbal signals. Lead II electrocardiogram was then recorded continuously at a speed of 25 mm/s for 60 seconds while the subject breathed as instructed (Cardiart 108T/MK-VII, BPL Ltd. Bangalore, Karnataka, India). The R-R intervals were measured accurately. The change in heart rate was calculated as the difference between the shortest and the longest R-R interval. HRV was expressed as beats per minute.[7] Impaired HRV was defined based on age-related normal value.[8]

The subjects performed the Valsalva manoeuvre by blowing into a mouthpiece connected to a modified sphygmomanometer and holding it at a pressure of 40 mmHg for 15 seconds while a continuous electrocardiogram was recorded. The result was expressed as the Valsalva ratio, which was the ratio of the

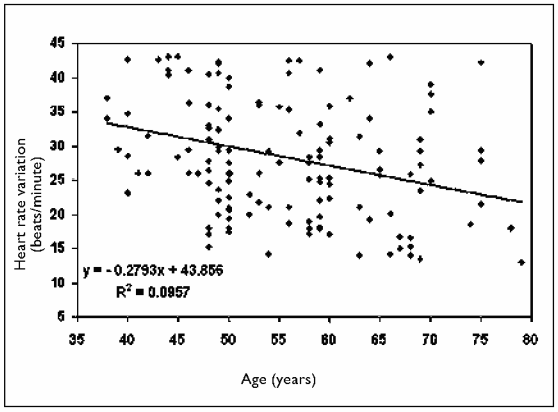


Fig. 1 The effect of age on heart rate variation with deep breathing in healthy subjects. y = -0.27x + 43.85; r = -0.32; p < 0.0001, where y = heart rate variation in (beats/minute) and x = age (years).

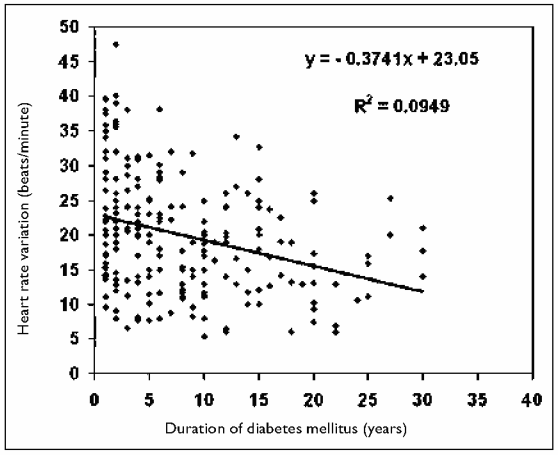


Fig. 2 Correlation between duration of diabetes mellitus and heart rate response to deep breathing. y = -0.37x + 23.05; r = -0.03; p < 0.0001, where y = heart rate variation (beats/minute) and x = duration of diabetes mellitus (years).

longest R-R interval after the manoeuvre to the shortest R-R interval during the manoeuvre, measured from the electrocardiogram trace. A Valsalva ratio of less than 1.2 was considered as impaired. Immediate heart-rate response to standing test was performed with the subject lying quietly on a couch while the heart rate was recorded continuously on an electrocardiograph. The subject was then asked to stand up unaided, and the point at starting to stand was marked on the electrocardiogram. The shortest R-R interval at or around the 15th beat, and the longest R-R interval at or around the 30th beat after starting to stand, were measured. The characteristic heart rate response was expressed by the 30:15 ratio. A 30:15 ratio of less than 1.03 was considered as impaired.[1] Systolic and diastolic blood pressures were recorded in the supine, and then in the standing position with an interval of at least two minutes between positions. A sustained drop in systolic (> 20 mmHg) or diastolic (> 10 mmHg) blood pressure after standing for at least two minutes was considered as having orthostatic hypotension.[9]

The data analysis was done by employing suitable statistical tests. Unpaired Student's *t*-test was employed to compare two independent groups. Analysis of variance was employed to compare more than two samples. Chi-square test was employed when proportions had to be compared. The measure of association between two continuous variables was tested using correlation coefficient. Multiple regression was employed to find the independent predictors of HRV among the varied baseline characteristics of study subjects and controls. Level of significance was measured by two-tailed test. The various analyses were performed using the Statistical Package for Social Sciences version 11.0 (SPSS India, Bangalore, Karnataka, India) and Statistical Package for Social Sciences for Windows version 11.0.1 (SPSS Inc, Chicago, IL, USA). Statistical significance was taken to be at p-value less than 0.05.

RESULTS

Typical symptoms of autonomic neuropathy observed in the study group included impotence in four subjects, persistent nocturnal diarrhoea in two subjects and abnormal pattern of sweating in two subjects. Orthostatic hypotension was observed in two subjects. The mean age was significantly higher with increasing duration of diabetes mellitus (mean age was 49, 52, 57, 58 and 61 years in study subjects having diabetes mellitus for a duration of ≥ 1, 2–5, 6–10, 11–15 and ≥ 16 years, respectively, $p < 0.0001$). Incidence of hypertension, stable angina and somatic neuropathy was significantly higher with advancing age: the proportion of diabetic patients with hypertension was found to increase from 21.42% (below 40 years of age) to 69.69% (above 60 years of age), $p < 0.0001$; the proportion of diabetic patients with stable angina increased from 10.71% (below 50 years of age) to 25.75% (above 60 years of age), $p < 0.0001$; and the proportion of diabetic patients with somatic neuropathy increased from 7.14% (below 40 years of age) to 73.21% (above 60 years of age), $p < 0.0001$. Additionally, the presence of somatic neuropathy was significantly higher in patients having foot ulcer, retinopathy and myocardial infarction (92.9%, 87.9% and 78.1%, respectively).

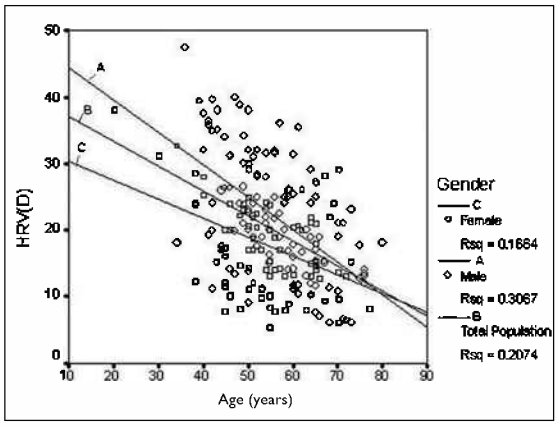
The patients in the study group were comparable, with respect to age and gender distribution, to the subjects in the control group. Diabetic patients had a slightly higher body mass index. The duration of diabetes mellitus was 8.02 ± 7 years. Study subjects had significantly higher blood pressure, resting heart rate and plasma blood sugar level compared to controls (Table II). The mean and standard deviation of HbA1c measured in 38 diabetic patients was 9.33% ± 3.73%. Table II also summarises the findings of the deep breathing test. HRV was significantly less in the study group compared to the control group. Both male and female patients with diabetes mellitus

had significantly lower HRV compared to their healthy counterparts. Reproducibility of the HRV was tested in 20 study and 20 control subjects on three different occasions. The values obtained were consistent in all the study subjects and in 17 control subjects. In three of the control subjects, the difference observed was 1–2 beats/minute. In the control subjects, negative correlation was found between age and HRV (Pearson's correlation coefficient, r = -0.32, $p < 0.001$; Fig.1). Age was an independent predictor of reduced HRV in control subjects (standardised coefficient, $\beta = -0.342$, $p < 0.0001$). In the healthy female subjects, HRV was less compared to that of males (27.02 ± 8.21 vs. 29.31 ± 8.85). Female gender was a significant determinant of reduced HRV ($\beta = -0.163$, $p = 0.044$). Body mass index, resting heart rate and blood pressure were not significantly associated with HRV.

The study group was divided into different subgroups according to complications and drug therapy. The number of diabetic patients, the mean and standard deviation of HRV in each subgroup, along with the p-value, are shown in Table III. HRV was lower in diabetic patients having hypertension, coronary artery disease, retinopathy, foot ulcer and somatic neuropathy, compared to diabetic patients free from these complications (Table III). HRV was significantly lower in female diabetic patients compared to male diabetic patients (The mean HRV in female diabetic patients was 17.66, while in male diabetic patients, it was 21.95, $t = 3.79$, $p < 0.0001$). HRV was lower in the β-blockers group, compared to the non-β-blockers group (Table III). No significant differences were observed in the HRV among the diabetic subjects on other drug therapies with or without complications (Table III). Negative correlation was observed between the duration of diabetes mellitus and HRV (Fig. 2). There was a negative correlation between age and HRV in study subjects (Fig. 3). HRV showed a trend of decline with severity of somatic neuropathy (Fig. 4). A positive correlation was found between the resting heart rate and HRV in diabetic patients, unlike in control subjects (Fig. 5). Body mass index, diastolic blood pressure, plasma blood sugar level, HbA1c and microalbuminuria were not significantly associated with HRV in study subjects.

The following baseline characteristics of the study group were entered into a multiple regression model: age, gender, duration of diabetes mellitus, body mass index, blood pressure, resting heart rate, status of hypertension, coronary artery disease, retinopathy, foot ulcer, somatic neuropathy, microalbuminuria, fasting blood sugar, postprandial blood sugar, blood sugar lowering agents and antihypertensive drugs. Among these baseline characteristics, presence of somatic neuropathy, age and female gender were the independent predictors of reduced HRV. Independent positive correlation was found between resting heart rate and HRV (Table IV). The clinical characteristics which showed significant association with HRV in the unpaired *t*-test and Pearson's correlation coefficient test, but were not independent predictors of HRV, are also provided in Table IV.

There were 47 diabetic patients free of all



HRV(D) = 48.583 - 4.88 *Gender - 0.392* age

Fig. 3 The effect of age on heart rate variation with deep breathing in female, male and total populations. y = - 4.88x + 48.58; gender - 0.39x; r = -0.44 for total population; $p < 0.0001$, where y = HRV(D) = heart rate variation (beats/minute) and x = age (years).

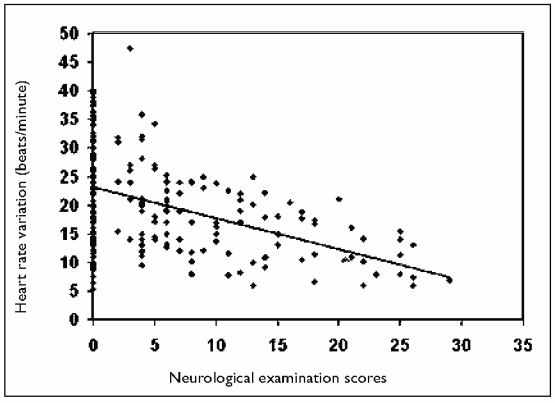


Fig. 4 The correlation between severity of somatic neuropathy and heart rate variation with deep breathing in study group. y = -0.54x + 23.11; r = -0.45; $p < 0.0001$, where y = heart rate variation (beats/minute) and x = severity of somatic neauropathy in numerial scores.

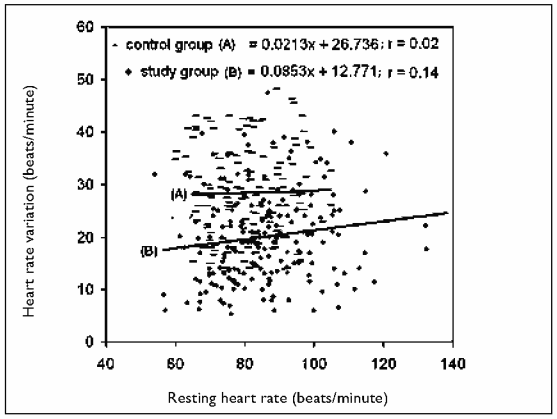


Fig. 5 The correlation between resting heart rate and heart rate variation with deep breathing in control and study subjects. y = 0.021x + 26.73; r = 0.02; p = NS in controls, y = 0.08x +12.77; r = 0.14; p = 0.02 in study subjects, where y = heart rate variation (beats/minute) and x = resting heart rate (beats/minute).

Table II. Characteristic features of control and study groups.

Variables	Control group (n = 141)	Study group (n = 207)	p-value
Age	55.21 ± 9.59	55.54 ±10.34	NS
Male/female ratio	87/54	115/92	NS
Body mass index (kg/m^2)	22.50 ± 3.04	23.21 ± 3.31	0.045
Systolic blood pressure (mmHg)	122.65 ± 10.71	140.53 ± 21.10	< 0.0001
Diastolic blood pressure (mmHg)	80.80 ± 4.31	85.74 ± 9.63	< 0.0001
Resting heart rate (beats/min)	79.79 ± 10.26	85.26 ± 13.99	0.0001
Fasting blood sugar (mg%)	85.54 ± 3.84	179.28 ± 70.02	< 0.0001
Postprandial blood sugar (mg%)	116.16 ± 4.14	233.87 ± 97.09	< 0.0001
HRV (beats/minute)	28.43 ± 8.66	20.04 ± 8.50	< 0.0001
In males*	29.31 ± 8.85	21.95 ± 8.89	< 0.0001
In females*	27.02 ± 8.21	17.66 ± 7.36	< 0.0001
Duration of diabetes mellitus (years)	nil	8.02 ± 7	
Any known clinical condition	nil	yes	

Data are expressed in mean ± SD where applicable.

HRV: heart rate variation with deep breathing. *sample size as shown in the male/female ratio.
n: sample size

complications (21 females and 26 males). Their mean age was 46.53 years. The mean duration of diabetes mellitus was 4.59 years. Comparison of mean HRV of these 47 cases with control subjects showed significant difference (26.19 vs. 30.93, t = 2.95, $p < 0.01$). In 50 of the study subjects and 30 of the control subjects, in addition to HRV, the heart rate responses to Valsalva manoeuvre and active standing were measured. This subset of the study group and control group were comparable with respect to age and gender distribution. HRV, Valsalva ratio and 30:15 ratio were lower in study subjects compared to control subjects (Mean HRV = 20.02 vs. 29, $p < 0.0001$; mean Valsalva ratio = 1.27 vs. 1.40, $p < 0.01$; mean 30:15 ratio = 1.08 vs. 1.10, $p < 0.01$). Of the 50 diabetic patients, 20 had impaired HRV, 16 had impaired Valsalva ratio and ten had impaired 30:15 ratio. Diabetic patients with normal HRV (n = 30) had normal values of Valsalva ratio and 30:15 ratio as well. Conversely, the diabetic patients with impaired Valsalva ratio and 30:15 ratio had impaired HRV as well. Of the ten diabetic patients with an impaired 30:15 ratio, only six had an impaired Valsalva ratio.

DISCUSSION

Vagal nerve traffic cannot be measured directly in humans. The assessment of HRV has thus become the most widely-used indirect measure of cardiac vagal function. Several workers have investigated the determinants of HRV in diabetes mellitus. But no common agreement in their study findings is observed. This study looked into the independent predictors of HRV among the clinical characteristics of type 2 diabetes mellitus. In the present study, the resting heart rate in the study group was higher compared to controls (Table II). Resting heart rate is generally considered to be under vagal tone.[5] Therefore, a higher heart rate observed in diabetic subjects is usually attributed to the parasympathetic dysfunction. Yet we did not find significant correlation between resting heart rate and HRV in healthy subjects (Fig. 5). Furthermore, in diabetes mellitus, an independent positive correlation was observed between resting heart rate and HRV (Fig. 5, Table IV). Thus, it appears that parasympathetic dysfunction alone may not contribute to the higher resting heart rate observed in diabetic patients.

The effect of ageing on HRV is well known. All the studies involving to date have found progressive reduction in the response with increasing age.[10-12] Our data based on 141 control subjects and 207 patients with type 2 diabetes mellitus were similar (Figs.1 & 3). The tests of the Ewing battery, which were developed with their normal ranges before this was appreciated, contained only one normal range, regardless of age.[1] Thus, they stand to generate false negative results among younger patients and false positive results among the older age group. Normal values reported by later workers for each age range of heart rate change are: 10–40 years: > 18 beats/minute; 41–50 years: >16 beats/minute; 51–60 years: > 12 beats/minute; and 61–70 years: > 8 beats/minute.[8] When these values were applied to our subjects, none of the subjects from the control group were found to have abnormal HRV. In the study group, 35 out of 207 diabetic patients had impaired HRV; but only 15 diabetic patients had a HRV of less than 10 beats/minute. Accordingly, we emphasise that age-

Table III. Effects of complications and medication on heart rate variation with deep breathing in the study group.

Effects	Heart rate variation (mean ± SD)		*t*-value	p-value
	With complications/medication	Free of complications/medication		
Hypertension	18.26 ± 7.58 (n = 105)	21.88 ± 9.03 (n = 102)	3.79	< 0.001
Stable angina	16.17 ± 6.10 (n = 25)	20.57 ± 8.66 (n = 182)	3.19	< 0.01
Myocardial infarction	17.06 ± 7.10 (n = 32)	20.59 ± 8.64 (n = 175)	2.49	< 0.05
Retinopathy	17.03 ± 6.32 (n = 58)	21.21 ± 8.96 (n = 149)	3.77	< 0.001
Foot ulcer	15.73 ± 7.06 (n = 28)	20.72 ± 8.52 (n = 179)	3.37	< 0.001
Somatic neuropathy	16.65 ± 6.42 (n = 104)	23.47 ± 8.98 (n = 103)	6.28	< 0.0001
Microalbuminuria	18.38 ± 8.19 (n = 28)	20.44 ± 8.56 (n = 179)	1.38	NS
Oral hypoglycaemic agents	19.48 ± 8.34 (n = 161)	22 ± 8.86 (n = 46)	1.72	NS
Insulin	18.45 ± 7.41 (n = 57)	20.65 ± 8.83 (n = 150)	1.8	NS
Beta blockers	17 ± 8.01 (n = 41)	20.8 ± 8.47 (n = 146)	2.68	< 0.01
ACE inhibitors	18.09 ± 7.54 (n = 37)	20.47 ± 8.66 (n = 170)	1.69	NS
Diuretics	16.87 ± 8.99 (n = 16)	20.31 ± 8.43 (n = 191)	1.47	NS
Calcium channel blocker	20.33 ± 7.91 (n = 20)	20.08 ± 8.55 (n = 187)	0.13	NS

n: sample size

related normal values are very relevant in diagnosis of autonomic dysfunction in patients with diabetes mellitus.

This study demonstrates gender-related differences in HRV in healthy and diabetic subjects. However, this difference is more marked in diabetic patients. Females had slightly less HRV compared to males in the control group. This finding is not in conformity with previous studies, which reported that there were no gender difference in HRV in healthy subjects.[6,13] With regard to whether either gender is more likely to develop autonomic dysfunction in diabetes mellitus, the literature has revealed conflicting reports. In the Diabetes Control and Complication Trial (DCCT), the presence of autonomic neuropathy correlated with male gender, along with age and duration of diabetes mellitus.[14] Jaffe et al showed that the male gender is predictive of depressed HRV, in addition to age, duration of diabetes mellitus, and retinopathy.[15] However, in another study of type 1 diabetic individuals, the female gender was found to be an independent determinant of autonomic dysfunction.[16] Toyry et al also observed diminished parasympathetic function in female patients with type 2 diabetes mellitus.[17] In the present study, female diabetic patients had significantly less HRV compared to their male counterparts. The female gender was one of the independent predictors of reduced HRV. Hence, our data also suggests that female diabetic patients are more prone to parasympathetic dysfunction than male diabetic patients.

In this study, although the correlation studies demonstrated that the duration of diabetes mellitus is associated with reduced HRV (Fig. 2), multiple regression analysis did not find that the duration of diabetes mellitus to be an independent predictor of reduced HRV (Table IV). Findings of previous studies on the relationship between the duration of diabetes mellitus and autonomic dysfunction are not consistent. Gundersen and Neubauer[18] and O'Brien et al[10] found an association between the duration of diabetes mellitus and reduced HRV in patients with type 1 diabetes mellitus unselected with respect to diabetic complications. Dyrberg et al also observed that diabetic neuropathy increased in frequency with duration of diabetes mellitus.[19] On the other hand, in Straub et al's study involving patients with type 1 and type 2 diabetes mellitus, the duration of diabetes mellitus did not correlate with the results of cardiovascular tests.[20] Similarly, Veglio et al did not find any correlation between the duration of diabetes mellitus and HRV in type 2 diabetic patients.[21] Furthermore, Lehtinen et al detected an impaired HRV in patients with newly-diagnosed type 2 diabetes mellitus.[22] Pfeifer et al detected a diminished autonomic dysfunction in patients with type 1 and type 2 diabetes mellitus shortly after the diagnosis of diabetes mellitus.[23] In this study, the study group was unselected with respect to age and diabetic complications. The age of patients with diabetes mellitus, along with proportion of study subjects with peripheral neuropathy, was significantly higher with increasing duration of diabetes

Table IV. Independent predictors of heart rate variation with deep breathing in the study group.

Predictors	Unstandardised coefficients		Standardised coefficients	*t*-test	Significance
	β	Std error	β		
Age	-0.225	0.06	-0.276	-3.76	0.000
Gender	-5.027	1.054	-0.295	-4.769	0.000
Resting heart rate	8.661×10^{-2}	0.037	0.143	2.351	0.02
Duration of diabetes mellitus	-0.133	0.085	-0.11	-1.55	0.12
Hypertension	-1.79	1.46	-0.106	-1.224	0.22
Stable angina	3.84	2.88	0.14	1.33	0.18
Myocardial infarction	3.35	2.77	0.14	1.20	0.22
Retinopathy	-0.53	1.34	-0.028	-0.39	0.69
Foot ulcer	0.139	1.68	0.006	0.083	0.93
Somatic neuropathy	-0.374	0.098	-0.318	-3.79	0.000
Beta blockers	0.66	1.6	0.031	0.412	0.681

mellitus. Hence, the negative correlation observed between the duration of diabetes mellitus and HRV in this study could largely be due to the negative impact of advancing age and presence of diabetic complications, in particular somatic neuropathy on HRV. Thus, we speculate that the duration of diabetes mellitus alone may not be an independent determinant of reduced HRV.

In this study, the hypertensive diabetic group had a lower HRV compared to the normotensive diabetic group. However, no independent association between hypertension and reduced HRV was observed (Table III). In the study group, an increasing trend in the proportion of hypertensives with age was observed. Therefore, the lower HRV found in the hypertensive diabetic group could be largely due to the negative impact of age. Takahashi et al also did not find a difference in HRV between hypertensive type 2 diabetic patients and normotensive type 2 diabetic patients.[24] Nevertheless, our study finding does not conform with the findings of Maser et al,[16] where hypertension was an independent determinant of reduced HRV in diabetic patients. It is difficult to explain the difference between the two studies. In this study, the study subjects had long-term type 1 diabetes mellitus. According to the recent studies, the pathogenesis of neuropathy varies between type 1 and type 2 diabetes mellitus.[25] We presume that the difference in pathogenesis of the two types of diabetes mellitus might have led to the difference observed between the present study and the study by Maser et al.

In this study, lower HRV was found in diabetic patients with coronary artery disease when compared to diabetic patients free of this complication (Table III). This study finding is consistent with the findings of Airaksinen et al.[26] However, this study did not find an independent association between coronary artery disease and reduced HRV (Table IV). Kronert et al did not find an association between coronary artery disease and reduced HRV in diabetic patients older than 50 years of age.[27] In our study group, the proportion of diabetic patients with stable angina increased from none (below 40 years of age) to 25.75% (above 60 years of age). Among the 31 diabetic patients with myocardial infarction, somatic neuropathy was detected in 25 (80.6%). Therefore, the reduced HRV observed in diabetic patients with coronary artery disease may be attributed to the influence of advancing age of the patients and presence of somatic neuropathy.

Diabetic patients with retinopathy had lower HRV compared to diabetic patients free of retinopathy (Table III). This finding is similar to the findings of previous investigators.[28,29] Even so, in this study, retinopathy was not an independent predictor of reduced HRV (Table IV). This finding does not conform with the findings of Spallone et al; they had found an independent association between retinopathy and reduced HRV in type 1 diabetic patients, but not in type 2 diabetic patients.[30] In the present study, diabetic patients with foot ulcer had lower HRV compared to diabetic patients free of foot ulcer (Table III). However, no independent association between foot ulcers and reduced HRV was observed (Table III). Out of 28 diabetic patients with foot ulcer, 26 (92.9%) had clinical signs of somatic neuropathy. Hence, the association found between reduced HRV and foot ulcer group could largely be due to the presence of somatic neuropathy.

This study not only demonstrates an independent association of somatic neuropathy with reduced HRV (Table IV), but also a progressive decline in HRV with severity of somatic neuropathy (Fig. 4). This finding

suggests a close association between somatic and parasympathetic nerve function abnormalities. The close association found between reduced HRV and somatic neuropathy in this study is consistent with the previous study findings.[31-33] However, HRV was significantly less in diabetic patients free of all complications compared to controls. This finding suggests that in some diabetic patients, parasympathetic dysfunction may either be isolated or may precede other complications.

There were certain limitations in this study. All the three parasympathetic function tests were not performed in all the subjects. One of the difficulties faced in conducting autonomic function tests was obtaining the patients' cooperation. Our study subjects were older and had multiple complications. Hence, several subjects could not hold the column of mercury at 45 mmHg for the requisite 10–15 seconds and could not stand up unaided from a lying position. This led to disturbances in the electrocardiogram signal and artifacts in the electrocardiogram trace. In contrast, all the subjects found the deep breathing test fairly simple and could perform as desired with some training. In this study, only one time domain method was employed to analyse the HRV. Other time domain and frequency domain methods were not employed due to a lack of suitable software in our clinical set-up at the time of this study. Glycated haemoglobin is considered to provide an accurate and objective measure of glycaemic control over a period of weeks and months. Due to financial constraints, it could not be measured in all the subjects. Therefore, the association between glycaemic control and HRV could not be studied satisfactorily. Our data suggests that the vagal nerve dysfunction mainly coexists with other peripheral neuropathies. But it may be isolated or precede the clinical detection of other complications of diabetes mellitus. Age and female gender are the other determinants of reduced HRV. Resting heart rate exerts an independent positive influence on HRV in type 2 diabetes mellitus.

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